Ca-amendment and tillage: Medium term synergies for improving key soil properties of acid soils

Clara Gómez-Paccard , Ignacio Mariscal-Sancho , Paloma León , Marta Benito , Pedro González , Rafaela Ordóñez , Rafael Espejo , Chiquinquirá Hontoria

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A B S T R A C T

Ca-amendments are routinely applied to improve acid soils, whilst no-tillage (NT) has been widely recommended in soils where traditional tillage (TT) has led to losses of organic matter. However, the potential interactions between the two treatments are only partially known. Our study was conducted on an annual forage crop agrosystem with a degraded Palexerult soil located in SW Spain, in order to assess if the combination of NT plus a Ca-amendment provides additional benefits to those of their separate use. To this end we analysed the effects of four different combinations of tillage and Ca-amendment on selected key soil properties, focusing on their relationships. The experimental design was a split-plot with four replicates. The main factor was tillage (NT versus TT) and the second factor was the application or not of a Ca-amendment, consisting of a mixture of sugar foam (SF) and red gypsum (RG). Soil samples were collected from 3 soil layers down to 50 cm after four years of treatment (2009). The use of the Ca-amendment improved pH and Al-toxicity down to 25 cm and increased exchangeable Ca^{2+} down to 50 cm, even under NT due to the combined effect of SF and RG. Both NT and the Ca-amendment had a beneficial effect on total organic carbon (TOC), especially on particulate organic carbon (POC), in the 0–5 cm layer, with the highest contents observed when both practices were combined. Unlike NT, the Ca-amendment failed to improve soil aggregation in spite of the carbon supplied. This carbon was not protected within the stable aggregates in the medium term, making it more susceptible to mineralization. We suggest that the fraction of Al extracted by oxalate from solid phase ($Al_{Oxa\text{-}Cu\text{-}K}$) and the glomalin-related soil proteins (GRSPs) are involved in the accumulation of carbon within water stable aggregates, probably through the formation of non-toxic stable Al-OM compounds, including those formed with GRSPs. NT alone decreased Al_K in the 0–5 cm soil layer, possibly by increasing POC, TOC and GRSPs, which were observed to play a role in reducing Al toxicity. From our findings, the combination of NT and Ca-amendment appears to be the best management practice to improve chemical and physical characteristics of acid soils degraded by tillage.

1. Introduction

Aluminium toxicity and Ca^{2+} deficiency are the main constraints for crop production in acid soils (Adams, 1984). Tillage, on the other hand, has been reported to decrease soil organic matter (SOM), thus affecting in turn other parameters related to soil quality (Reeves, 1997). The soils studied in our work (old Ultisols in the Cañamero's raña surface, SW of the Iberian Peninsula) suffer from both high acidity and degradation from tillage. Restoration of these degraded soils would require the use of a Ca-amendment to raise pH and alleviate Al toxicity, as well as the introduction of soil conservation practices such as no-tillage (NT).

Lime and gypsum amendments or the combination of both (Ca-amendment) are widely used to solve problems of soil acidity (Oates and Kamprath, 1983; Shainberg et al., 1989; Peregrina et al., 2006). Lime provides Ca^{2+} and generates OH^- ions that neutralize the acidity, thus raising the pH. This leads to the precipitation of aluminium from the soil solution in the form of insoluble hydroxides (Bohn et al., 1985). Lime is fairly insoluble, and thus to correct aluminium toxicity in deeper horizons gypsum addition is recommended due to its higher solubility (Reeves and Sumner, 1972). Gypsum delivers Ca^{2+} to the deeper horizons, increasing Ca^{2+} saturation in the exchange complex (Shainberg et al., 1989). At the same time it favours the formation of the non-toxic ionic pars $AlSO_4^+$ in the soil solution (Kinraide and Parker, 1987), thus

reducing the activity of the toxic Al^{3+}. The increase of the Ca/Al ratio in the soil solution also helps to decrease the activity of the toxic Al^{3+} (Noble et al., 1988) while the sorption of SO_4^{2-} to the soil matrix liberates OH^- ions that raise the pH. This latter process is known as the "self liming effect" (Reeves and Sumner, 1972).

Whereas the effects of these amendments on pH, Al toxicity and biomass production are well documented (Pavan et al., 1982; Oates and Kamprath, 1983; Shainberg et al., 1989; Fageria and Baligar, 2008), their effects on SOM are contradictory (Haynes and Naidu, 1998). Variations in SOM content due to changes in soil management are normally slow and, therefore, monitoring changes on shorter time scales requires the selection of organic matter fractions that are easily degraded and mineralized. Such fractions include particulate organic matter (POM), which represents a labile fraction of the SOM and consists of particles between 0.053 and 2 mm (Cambardella and Elliott, 1992). We would expect Ca-amendments to promote the accumulation of SOM through the enhancement of biomass production (both root and above-ground biomass) as a result of a reduction of Al toxicity and nutrient supply (Haynes and Naidu, 1998). However, if the Ca-amendment also promotes microbial activity that effect may be counteracted by a higher SOM mineralization rate (Fuentes et al., 2006). In fact some authors (Marschner and Wilczynski, 1991; Chan and Heenan, 1999) have found lime to decrease organic carbon content, but the effect only occurs during the first few years following the application and vanishes with time (Caires et al., 2006).

Another important aspect is the effect of the Ca-amendments on arbuscular mycorrhizal fungi (AMF), which have been reported to increase the access of plant roots to limiting nutrients and to enhance stress resistance in acidic environments (Cumming and Ning, 2003). AMF are also responsible for the production of glomalin, a glycoprotein that has been reported to play a role in soil aggregation (Wright and Upadhyaya, 1998) and to help reduce Al toxicity in acid soils (González-Chávez et al., 2004; Etcheverría, 2009; Aguilera et al., 2011). When referring to glomalin we use the term glomalin-related soil protein (GRSP) proposed by Rillig (2004), since some other heat-stable proteins of non-AMF origin may be extracted in the process (Rosier et al., 2006), although GRSPs are largely of AMF origin (Rillig, 2004). We also distinguish between the total fraction (GRSP) and the easily extractable fraction (EE-GRSP), which is supposed to represent recent deposits (Wright and Upadhyaya, 1998). Soil management practices influence AMF, thus modifications of the soil pH can alter the distribution of AMF species (Abbott and Robson, 1977) and spore production (Raznikiewicz et al., 1994), although in the study by Wang et al. (1993) lime hardly affected root colonization. To our knowledge, no studies have reported the direct effects of Ca-amendments on GRSPs.

The effect of the Ca-amendments on soil aggregation is also unclear (Haynes and Naidu, 1998). It would be expected to have a favourable effect by increasing the amount of residual biomass (Tisdall and Oades, 1982), adding calcium (Chan and Heenan, 1999), favouring the precipitation of aluminium as hydroxides (El-Swaify and Emerson, 1975) and promoting biological activity, which results in the production of polysaccharides and other microbially-derived binding agents (Six et al., 2004). However, it can also have adverse effects, such as the dispersion of clay by repulsion as a result of increasing negative charges in the exchange complex due to the pH increase and reduced Al^{3+} activity (Haynes and Naidu, 1998). Increased SOM mineralization as a consequence of the stimulated microbial activity (Haynes and Swift, 1988) could also reduce aggregate stability in the short term (Chan and Heenan, 1999). These opposing effects can explain the contradictory results obtained in both laboratory and field studies. These studies have reported positive effects (Chan and Heenan, 1999; Briedis et al., 2012b), negative effects (Roth and Pavan, 1991; Koutika et al., 1997; Westerhof et al., 1999), variable effects depending on soil type (Castro and Logan, 1991) and even insubstantial effects (Stenberg et al., 2000).

With regards to tillage, its negative impact on SOM is mainly due to (i) aeration enhancing mineralization (Reicosky et al., 1995) and (ii) the mechanical breakdown that exposes the SOM that was previously physically protected within the aggregates against microbial degradation (Six et al., 2000). The benefits of NT on SOM have been described in a wide range of studies (Paustian et al., 1997; West and Post, 2002). No-tillage promotes the formation of macroaggregates and helps to stabilize and store soil carbon through the formation of microaggregates within the macroaggregates (Six et al., 1998). AMF have been reported to decrease with tillage mainly due to mechanical disturbances (Wright et al., 1999; Kabir, 2005), which cause the direct disruption of roots and hyphas, whereas NT practices favour fungal development (Kabir et al., 1998; Galvez et al., 2001). We would expect, therefore, that GRSPs are sensitive to management changes in the same direction. Indeed, a higher concentration of these proteins has been found in soils under NT compared with tilled ones (Borie et al., 2006; Curaqueo et al., 2011).

In addition, NT improves soil aggregation by stopping the mechanical breakdown and by the promotion of SOM (Paustian et al., 2000; Six et al., 2000). The POM fraction is, above all, reported to be important for the formation and stabilization of microaggregates within macroaggregates (Jastrow, 1996; Six et al., 2002). No-tillage also favours fungal development, which increases soil aggregation through hyphal trapping and the production of binding substances (Six et al., 2004). Such substances include GRSPs, which have been associated with aggregate stability since they are reported to act as a glue stabilizing aggregates, possibly due to their recalcitrant nature and hydrophobic characteristics (Wright and Upadhyaya, 1998; Rillig et al., 2002).

The increase of both SOM and GRSPs under NT would not only enhance soil aggregation, but could also help to alleviate Al toxicity. The role of SOM in this process has been widely reported (Hargrove and Thomas, 1981a; Haynes and Mokolobate, 2001; Wong and Swift, 2003) and has been attributed to (i) the increase of pH resulting from the decomposition of SOM, which leads to the precipitation of exchangeable and soluble aluminium, and (ii) the production of non-toxic stable complexes formed by the SOM and the aluminium. More recently, GRSPs have been reported to be a response by AMF to reduce Al toxicity in acid soils (Seguel et al., 2013). Due to their high cation exchange capacity and high affinity for polyvalent cations (Etcheverría, 2009), GRSPs have the capacity to sequester substantial quantities of Al by forming stable compounds (Aguilera et al., 2011).

The combination of NT with Ca-amendment, therefore, seems to be a suitable management approach for acid soils degraded by an excess of tillage, such as the soils studied here. Some studies have examined the combined use of these practices (Arshad et al., 1999; Stenberg et al., 2000; Conyers et al., 2003; Soon and Arshad, 2005), although they have only focused on a few variables and none of them have studied the combined use of lime and gypsum. Regarding the role of SOM in reducing Al toxicity, this has mainly been investigated in laboratory studies (Hargrove and Thomas, 1981b; Mokolobate and Haynes, 2002; Naramabuye and Haynes, 2007), and only occasionally in the field (Godsey et al., 2007; Brown et al., 2008). The role of GRSPs in reducing Al toxicity has only been reported recently and further research is needed to confirm its relevance under different conditions.

We hypothesized that NT could: (i) compensate the possible negative effects of the Ca-amendment on SOM content and soil aggregation; (ii) contribute to decrease aluminium toxicity through the enrichment of SOM and GRSPs. We sought to

determine if the combination of NT plus a Ca-amendment can provide additional benefits to those obtained when used separately. To this end, we examined the effects of (i) traditional tillage (TT) compared with no-tillage (NT), and (ii) the use or not of a Ca-amendment on selected soil properties, with special emphasis on their inter-relationships and effectiveness in increasing SOM content, reducing Al toxicity and promoting soil aggregation.

2. Materials and methods

2.1. Study site and experimental design

The study was conducted on an experimental plot established in October 2005 in the Cañamero's Raña (SW Spain). The average altitude of the study site is 580 m and its slope is less than 0.5%. The climate is moist Mediterranean (Csa), based on Köppen's classification. The mean annual temperature is 15.0 °C, the mean annual precipitation is 869 mm and the mean annual evapotranspiration (Penman–Monteith method) is 1248 mm. As a result of the subtropical climate that prevailed when these soils were formed in the middle Pliocene (Espejo, 1987) they have the following characteristics: Kaolinite as the dominant mineral in the clay fraction, low pH ranging from 5.1–5.3 at the surface to 4.4–4.6 at 150 cm depth, low content in exchangeable bases, Al-dominated exchange complex, low bio-available P content; as well as having a high content of rock fragments (Espejo, 1987; Espejo and Cox, 1992). According to Soil Taxonomy the soil is a clay-skeletal, kaolinitic, acid, thermic Plinthic Palexerult; Table 1 shows selected chemical and physical properties of the representative soil profile.

The experiment was established as a split-plot design with four replicates and a total of 16 plots, 4 m × 16 m each. The main factor was tillage: no-tillage (NT) versus traditional tillage (TT), and the second factor was the use or not of a Ca-amendment. The four treatments studied, therefore, were traditional tillage without Ca-amendment (TT + not amended), traditional tillage plus Ca-amendment (TT + amended), no-tillage without Ca-amendment (NT + not amended) and no-tillage plus Ca-amendment (NT + amended). The Ca-amendment consisted of a mixture of sugar foam (SF) and red gypsum (RG). We used a combination of these products because SF, a by-product from the sugar industry rich in $CaCO_3$, acts preferentially on the surface Ap horizon, whereas RG, a by-product from the titanium oxide production industry rich in $CaSO_2{\cdot}2H_2O$ affects the AB and Bt horizons due to its higher solubility. The SF was applied at a rate of 3.9 Mg dry product ha^{-1} and RG at a rate of 7.5 Mg ha^{-1}. We also added 100 kg MgO ha^{-1} in the form of converter basic slag (CBS) plus 100 kg MgO ha^{-1} in the form of $MgSO_4$ in order to compensate Mg losses from the exchange complex as a result of the RG addition (O'Brien and Sumner, 1988). The composition of the amendments is shown in Table 2, adapted from Peregrina et al. (2006, 2008).

The experimental area was cropped and tilled (disturbing the upper 20 cm of soil) from approximately 1940 to 1990, and then abandoned due to the declining productivity. A natural pasture and isolated specimens of *Halimium ocymoides* and *Cistus ladanifer* became established and the soil remained undisturbed for 15 years (Mariscal-Sancho et al., 2009). In September 2005, the natural vegetation was cleared throughout the experimental area using a cultivator (two passes) and a power tiller (one pass) that affected the upper 20 cm. The Ca-amendment was applied once only at the beginning of the experiment and it was incorporated into the top 0–7 cm soil layer using a power tiller in order to avoid losses due to the wind and to prepare the soil for seeding. The same management was applied in the unamended plots to keep the conditions identical. Following the establishment of the experiment, the NT plots were not ploughed again. The soil in the TT plots was disturbed every year down to 20 cm, using a cultivator (2 passes) for soil preparation and a power tiller for fertilizer incorporation and weed control. Weeds were controlled in the NT plots with glyphosate (36% purity) at a rate of 2.5 L ha^{-1} prior to seeding. Each year 36 kg N ha^{-1}, 92 kg P ha^{-1} and 92 kg K ha^{-1} were added to all plots in autumn. All plots were sown each autumn using a direct drill seeder with a mixture of *Avena sativa* (57%), *Tritico secale* (17%) and *Vicia sativa* (26%), at a rate of 140 kg ha^{-1}. Every year at maturity (July), the crop was harvested at a height of 20 cm above ground and crop residues were left on the soil.

Table 2
Chemical composition of the amendments.

Component	SF	RG	CBS
	g kg^{-1}		
CaO	437	231	395
SO_4^{2-}	5.1	465	nd
SiO_2	17.3	11.2	9.6
F^-	nd	nd	nd
P_2O_5	8.1	nd	0.23
Al_2O_3	24.2	12.3	nd
Na_2O	1.05	0.9	<0.8
K_2O	1.95	<0.5	nd
Fe_2O_3	1.31	38.8	21
TiO_2	nd	41.3	nd
MnO	<0.1	1.1	0.3
MgO	47.3	1.5	401
OM	86.7	nd	–
Lime*	765	nd	83
Active lime**	213	nd	nd
LI***	467.6	162	175

SF, sugar foam; RG, red gypsum; CBS, converter basic slag; and nd: not detected.
* Total calcium equivalent.
** Calcium carbonate reactive with ammonium oxalate.
*** Loss on ignition (100–1000 °C).

2.2. Soil sampling and analysis

In October 2009, after four years of the experiment, 3 disturbed soil samples from each plot were collected at depths of 0–5, 5–10, 10–25 and 25–50 cm. The samples from each plot and depth were combined, air-dried and passed through a 2 mm sieve. All biochemical and physical analyses were performed on the

Table 1
Selected properties of the representative soil profile.

Horizon (cm)	OM (%)	pH		Af^+ ($cmol_c$ kg^{-1})	Exchangeable bases				RF (%)	Texture		
		H_2O	KCl		Ca^{2+}	Mg^{2+}	Na^+	K^+		Sand	Silt	Clay
					$cmol_c$ kg^{-1}					(%)		
Ap (0–25)	3.38	5.3	4.3	0.76	1.12	0.21	0.05	0.65	52	80.1	6.1	13.8
AB (25–35)	1.59	5.0	4.0	1.01	0.85	0.16	0.04	0.08	47	69.1	5.6	25.3
Bt (35–90)	0.25	4.9	3.7	1.75	0.82	0.11	0.03	0.07	56	59.9	4.7	35.4
Btv (90–200)	nd	4.6	3.4	2.23	1.12	0.1	0.05	0.06	75	56	5.1	38.9

OM, organic matter; RF, rock fragments (>2 mm; mainly 2–30 cm); and nd, not determined.

composed samples in duplicate or triplicate. Soil pH was determined in de-ionized water (1:2.5 soil/water ratio). Exchangeable calcium was extracted with ammonium acetate at pH 7 and then determined by atomic absorption spectrophotometry. An adaptation of the method of Soon (1993) was used to extract various forms of aluminium from the solid fraction. Extracts were obtained by shaking (i) 3 g of soil with 30 mL of 1 M KCl for 30 min (Al_K); (ii) 3 g of soil with 30 mL 0.1 M $CuCl_2$ + 0.5 M KCl for 1 h (Al_{Cu}); and (iii) 0.25 g of soil with 0.2 M ammonium oxalate at pH 3.0 in the dark for 4 h (Al_{Oxa}). Aluminium in the extracts was determined by atomic absorption spectrophotometry. Following Álvarez et al. (2009) and Vieira et al. (2008) we assumed that (i) Al_K represents exchangeable Al^{3+}, (ii) the difference between Al_{Cu} and Al_K (Al_{Cu-K}) represents the Al-OM compounds with low to medium stability, and (iii) Al_{Oxa} minus Al_{Cu-K} ($Al_{Oxa-Cu-K}$) represents the amorphous Al plus the highly stable Al-OM compounds plus the Al from the octahedric layer of 2:1 clay mineral. In order to assess changes in SOM we determined TOC by the Walkley-Black method and POC according to Cambardella and Elliott (1992). Water-stable and unstable aggregates (WSA, WUA) were determined by wet-sieving of air-dried 1–2 mm aggregates through a 250 μm sieve (Kemper and Rosenau, 1986). $WSA_{1-2\ mm}$, in fact the percentage of water-stable macroaggregates, was calculated as the weight of stable aggregates divided by the sum of stable and unstable aggregates. Organic carbon was determined in both aggregate fractions (C-WSA and C-WUA). EE-GRSP was extracted with 20 mM sodium citrate at pH 7.0 and 121 °C for 30 min and GRSP was extracted with 50 mM sodium citrate at pH 8.0 and 121 °C in sequential cycles of 1 h (Wright and Upadhyaya, 1998) until the supernatant was straw-coloured (10–12 cycles). Soil protein in the extracts was quantified in duplicate, using the Bradford protein assay with bovine serum albumin as the standard.

Data were analysed statistically with the general linear model (GLM) ANOVA for a split-plot design and simple and multiple regressions were applied in the software Statgraphics Centurion XVI.

3. Results

3.1. Total and particulate organic carbon and glomalin-related soil proteins

Table 3 shows that after 4 years, tillage and the Ca-amendment only influenced TOC and POC in the top soil layer (0–5 cm). NT increased TOC by 19% with respect to TT in the same layer. Application of the Ca-amendment had a less marked effect, which was, however, enhanced by the NT treatment (Fig. 1). TOC content decreased in the following order: NT + amended (39.9 g kg^{-1}) > NT + not amended (36.8 g kg^{-1}) > TT + amended (32.8 g kg^{-1}) = TT + not amended (31.5 g kg^{-1}). POC was more sensitive to tillage than TOC; thus in the NT plots, POC and TOC contents were a 44% and a 19% higher, respectively than in the TT plots. We also found a positive effect of the Ca-amendment on POC with a difference of 15% between amended and unamended plots. POC decreased in the following sequence: NT + amended (16.0 g kg^{-1}) > NT + not amended (13.7 g kg^{-1}) > TT + amended (10.9 g kg^{-1}) > TT + not amended (9.7 g kg^{-1}).

As shown in Table 3, EE-GRSP was more sensitive to the treatments than GRSP. Only tillage had an effect on these proteins in the 0–5 cm layer. NT plots had higher average EE-GRSP and GRSP contents than TT (4% and 8%, respectively). In the 5–10 cm layer the Ca-amendment reduced the EE-GRSP, with values of 2.14 mg $g^{-1}$for the amended plots compared with 2.23 mg $g^{-1}$for 1for the unamended ones. No similar effect on GRSP content or interactions between treatment factors was observed.

3.2. pH and Ca

The Ca-amendment significantly raised pH down to a depth of 25 cm (Table 4). The effect was especially marked in the 0–5 and 5–10 cm soil layers, where the pH increased by nearly one unit. By contrast, the increase in the 10–25 cm layer was only 0.5 units and insubstantial at greater depths. There was a weak ($p < 0.1$) interaction between tillage and Ca-amendment in the 0–5 cm layer. In this layer, the pH in the amended plots was higher for NT than for TT (6.3 vs 6.0, Fig. 2). Tillage also influenced the 10–25 cm layer where pH was slightly higher ($p < 0.1$) for TT than for NT plots (5.2 vs 4.9). No effect on pH was observed at greater depths (25–50 cm layer).

The distribution of Ca with depth followed a similar trend as the pH (Table 4 and Fig. 2), but in this case the Ca-amendment increased Ca content down to 50 cm. The differences between amended and unamended plots were especially marked in the 0–10 cm layer, with values up to four times greater in the amended plots, and still being double at a depth of 10–25 cm. Tillage influenced Ca content down to 25 cm in the amended plots. NT plots had higher Ca contents in the 0–5 cm layer than TT (6.4 vs 5.3 $cmol_c\ kg^{-1}$, $p < 0.1$, Fig. 2). As for pH, the Ca content displayed a different pattern deeper in the soil (5–25 cm) with the TT + amended plots containing more Ca than their untilled counterparts (NT + amended). Tillage had no effect on the Ca content in the unamended plots.

3.3. Al forms in the solid fraction

The Ca-amendment had a strong effect on Al_K down to a depth of 25 cm in both NT and TT plots. In the top 10 cm, Al_K was completely removed and from 10 to 25 cm the content in the amended plots was reduced to approximately a third of that in the unamended plots (0.31 vs. 0.83 $cmol_c\ kg^{-1}$) (Table 4 and Fig. 2). The differences between amended and unamended plots in the deepest layer were not statistically significant. An interaction between the two factors occurred in the 0–5 cm layer, such that NT in the unamended plots reduced the Al_K content to almost the half with respect to TT (0.32 vs 0.56 $cmol_c\ kg^{-1}$). No significant effect of tillage on Al_K was observed in deeper layers. Unlike Al_K, Al_{Cu-K} was not affected by the Ca-amendment. The 5–10 cm soil layer in the NT plots had higher Al_{Cu-K} levels than in the TT plots (0.94 vs 0.76 $cmol_c\ kg^{-1}$). The $Al_{Oxa-Cu-K}$ content displayed the opposite trend to Al_K; namely, $Al_{Oxa-Cu-K}$ levels were higher in the amended plots than in the unamended plots (20% higher in the 0–5 cm layer, 16% in the 5–10 cm layer and 13% in the 10–25 cm layer). The $Al_{Oxa-Cu-K}$ content down to 10 cm was increased by NT as follows: in the 0–5 cm layer it was higher in NT + amended (19.8 $cmol_c\ kg^{-1}$) compared with TT + amended (17.4 $cmol_c\ kg^{-1}$), 1), and in the 5–10 cm layer it was higher in NT (20.2 $cmol_c\ kg^{-1}$) compared with TT (18.9 $cmol_c\ kg^{-1}$).

3.4. Aggregate stability and organic C associated with water-stable macroaggregates

Aggregate stability was influenced by tillage but not by the Ca-amendment (see Table 3). The effect was significant in the top layer (0–5 cm), with $WSA_{1-2\ mm}$ values approximately 10% higher in the NT plots than in the TT plots. Although this difference is not large, it is substantial considering the intrinsic high stability of the soils studied. The difference in $WSA_{1-2\ mm}$ in the 5–10 cm layer amounted to 5%, whereas in the 10–25 cm layer there was no difference. Water-stable macroaggregates in the top soil layer (0–5 cm) had significantly larger ($p < 0.05$) organic C contents relative to water-unstable aggregates (data not shown) for all the treatments, but especially for NT (12% higher). We observed an

Table 3
Effect of tillage (T) and Ca-amendment (A) on total and particulate organic carbon (TOC and POC, respectively), easily extractable and total glomalin-related soil proteins (EE-GRSP and GRSP, respectively), percentage of water-stable macroaggregates ($WSA_{1-2\,mm}$) and organic C associated with water stable macroaggregates (C-WSA).

Depth	Treatment	TOC ($g\,kg^{-1}$)	POC ($g\,kg^{-1}$)	EE-GRSP ($mg\,g^{-1}$)	GRSP ($mg\,g^{-1}$)	$WSA_{1-2\,mm}$ (%)	C-WSA ($g\,kg^{-1}$ WSA)
0–5 cm							
	Tillage						
	TT	32.2	10.3	2.26	11.18	82.2	31.0
	NT	38.3	14.8	2.35	12.02	91.6	34.6
	Ca-amendment						
	Not amended	34.2	11.7	2.30	11.61	86.8	32.4
	Amended	36.3	13.4	2.31	11.59	87.0	33.2
	Effects						
	T	**	**	**	*	**	ns
	A	**	**	ns	ns	ns	ns
	TXA	*	+	ns	ns	ns	+
5–10 cm							
	Tillage						
	TT	31.3	10.1	2.17	10.55	92.0	33.0
	NT	31.9	10.2	2.21	10.56	96.6	35.6
	Ca-amendment						
	Not amended	31.7	9.8	2.23	10.51	94.7	34.1
	Amended	31.5	10.6	2.14	10.60	93.9	34.5
	Effects						
	T	ns	ns	+	ns	*	ns
	A	ns	ns	**	ns	ns	ns
	TXA	ns	ns	ns	ns	ns	ns
10–25 cm							
	Tillage						
	TT	24.8	5.7	nd	nd	74.0	22.0
	NT	24.5	5.3	nd	nd	78.1	25.6
	Ca-amendment						
	Not amended	24.7	5.3	nd	nd	77.5	24.5
	Amended	24.5	5.7	nd	nd	74.6	23.1
	Effects						
	T	ns	ns			ns	ns
	A	ns	ns			ns	ns
	TXA	ns	ns			ns	ns
25–50 cm							
	Tillage						
	TT	7.7	2.7	nd	nd	nd	nd
	NT	7.3	2.5	nd	nd	nd	nd
	Ca-amendment						
	Not amended	8.3	2.8	nd	nd	nd	nd
	Amended	6.8	2.4	nd	nd	nd	nd
	Effects						
	T	ns	ns				
	A	ns	ns				
	TXA	ns	ns				

TT, traditional tillage; NT, no-tillage; ns, not significant; and nd, not determined.
$^{+}$ $p < 0.1$.
* $p < 0.05$.
** $p < 0.01$

interaction in the 0–5 cm layer: water-stable macroaggregates accumulated more C in NT + not amended (35.0 g kg^{-1}_{WSA}) than in TT + not amended (29.8 g kg^{-1}_{WSA}, Fig. 1).

4. Discussion

4.1. Organic carbon and glomalin-related soil proteins

The beneficial effect of NT on soil organic carbon content depends on time, climate, soil, crop, and other environmental factors and the effect has been reported to decrease with depth (West and Post, 2002; Soane et al., 2012). In our case, we found a higher content of both POC and TOC in the top layer (0–5 cm) of the NT plots compared with the TT plots. The larger increase in POC than in TOC is in agreement with Cambardella and Elliott (1992) who stated that POC was more sensitive to management changes, especially tillage, than TOC. Unlike other authors (Marschner and Wilczynski, 1991; Chan and Heenan, 1999), we found a positive influence of the Ca-amendment on both TOC and POC, although this improvement was restricted to the surface 0–5 cm layer. The highest carbon contents were obtained with the combination of the Ca-amendment and no-tillage (Fig. 1), suggesting that this combination is the best management practice for accumulating organic carbon in the surface soil layer. Briedis et al. (2012a) also found that TOC and POC was increased by lime addition in untilled plots and attributed this effect to higher biomass (above-ground and roots) production; however, as in our work, the effect was only significant in the 0–5 cm layer. Taking into account the small addition of organic matter provided by the sugar foam, we also attribute the increase in TOC and POC in the amended plots to an increased biomass production. As a result of the higher nutrient bioavailability and the reduced Al toxicity resulting from the Ca-amendment, the aboveground biomass production in the amended plots was higher throughout the experimental period (2005–2009) (data not shown). The average above-ground biomass production in the amended plots was 5190 kg ha^{-1} vs 3636 kg ha^{-1} in the

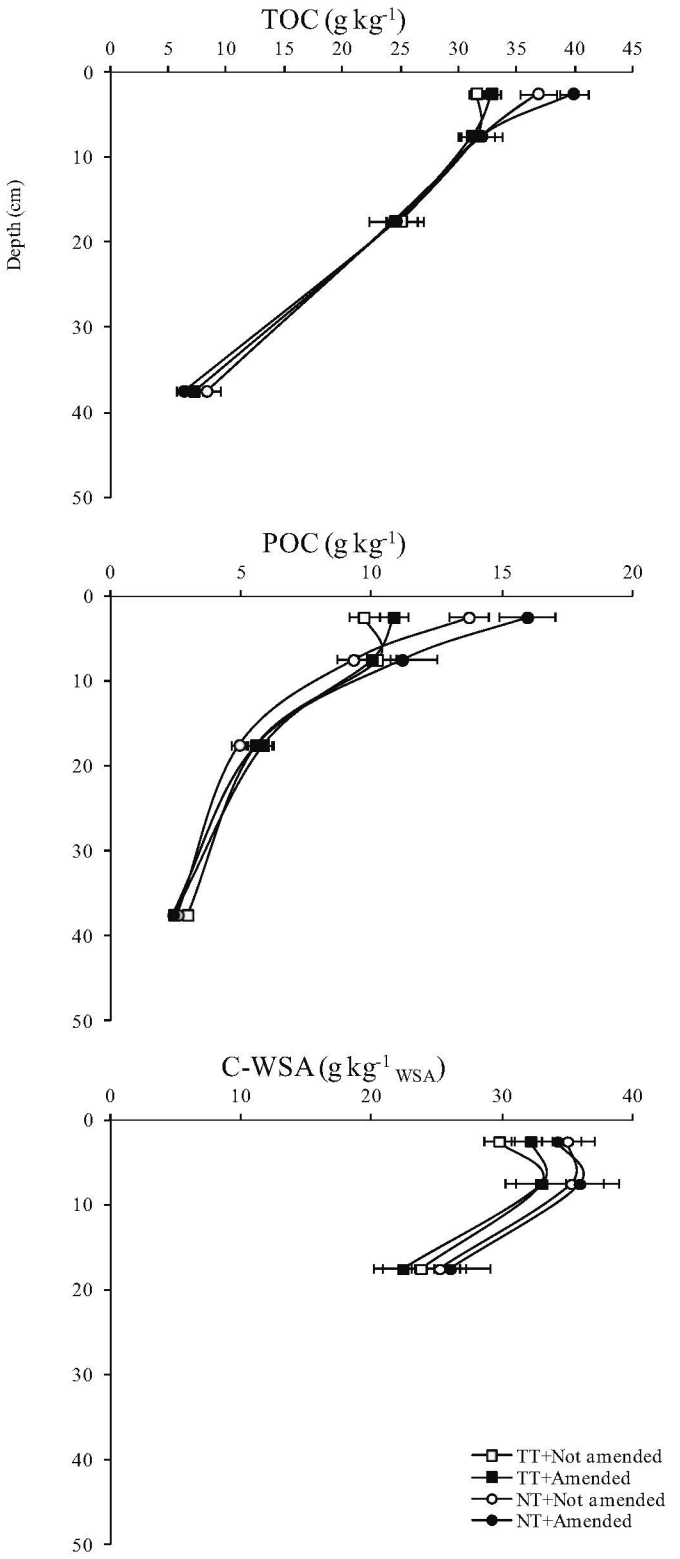

Fig. 1. Vertical profile of total organic carbon (TOC), particulate organic carbon (POC) and organic C associated with water stable macroaggregates (C-WSA). TT, traditional tillage; and NT; no-tillage. Bars represent standard error of the mean.

unamended plots. The reduction of aluminium toxicity must have also facilitated better root development in the amended plots (Foy, 1992) and, therefore, an additional input from plant residues.

Despite the larger average 2005–2009 above-ground biomass production in the TT + amended plots (4930 kg ha^{-1}) compared with TT + not amended (3311 kg ha^{-1}), differences in TOC and POC were not significant. These results suggest that tillage suppressed the beneficial effect of the Ca-amendment on organic carbon, possibly as a result of the destruction of aggregates, which exposed the SOM to mineralization (Cambardella and Elliott, 1992; Reicosky et al., 1997).

Considering the GRSP as AMF activity indicators (Wright and Upadhyaya, 1998; Lovelock et al., 2004), the decreased EE-GRSP and GRSP contents found in the TT plots could be attributed to damages to arbuscular mycorrhizal fungi (AMF) caused by tillage, as suggested by Wright et al. (1999) and Kabir (2005). Adverse effects of soil tillage on EE-GRSP and GRSP have also been observed previously by several authors (Borie et al., 2006; Hontoria et al., 2009). The effect of the Ca-amendment on GRSPs is still unknown but considering the production of GRSPs as a mechanism to protect against aluminum toxicity, we might expect a lower production of these proteins in the amended plots, as found in the 5–10 cm layer for EE-GRSP. The lack of response in the upper layer could be attributed to opposing effects that need to be researched further.

4.2. Soil acidity and exchangeable Ca

The effects of lime and gypsum on acidity have been widely studied and are known to change down the soil profile (Adams, 1984; Shainberg et al., 1989). Comparing the pH and Ca profiles in the amended versus the unamended plots, we found the greatest differences in the 0–5 and 5–10 cm layers, with smaller differences in the 10–25 cm layer. In these layers, the pH increase down to 25 cm was caused by the SF due to its high content in active or easily soluble lime, whereas the Ca increase was caused by both the $CaCO_3$ of the SF and the $CaSO_4{\cdot}2H_2O$ of the RG. In the 25–50 cm layer we only found differences in the Ca content and we attribute these differences to the $CaSO_4{\cdot}2H_2O$ of the RG, which due to its higher solubility can be transported by water down to this depth. Despite the self-liming effect of the gypsum, pH did not change in this deeper layer, probably because OH^- ions released from the soil matrix by sulphates were removed by H^+ and Al^{3+} that were displaced from the exchange complex by Ca^{2+} (the "salt effect" reported by Shainberg et al., 1989). This effect of gypsum has been reported by Garrido et al. (2003) and Peregrina et al. (2006) in laboratory studies carried out with soils from the same area, treated with RG.

In the amended plots, we observed that pH and Ca in the surface layer were higher in NT compared with TT, while below 5 cm they were higher in TT compared with NT, although the profiles with depth had the same form in both NT and TT. We attribute these differences and similarities in the profiles to particularities of the tillage treatments and the solubility of the Ca-amendment. Bearing in mind that at the beginning of the experiment, the Ca-amendment was incorporated in the upper 7 cm using a power tiller, the absence of tillage in the NT plots meant that most of the Ca-amendment remained in the surface layer, explaining the higher pH and Ca content. On the other hand, in the tilled plots, the cultivator contributed to partially incorporate the Ca-amendment in deeper soil layers, and thus below 5 cm we found higher pH and Ca levels. Although the cultivator used for tilling did not mix the soil layers thoroughly (Dickey, 1990) compared with a moldboard or a disk harrow, which homogenize the soil, a small amount of soil from the upper layer would have been incorporated into deeper layers. As a result the pH and Ca profile in the tilled plots below 5 cm was gradual and had a similar pattern to NT.

Despite the absence of tillage, the effect of the Ca-amendment in the NT plots was significant down to 25 cm for pH and down to 50 cm for Ca. Other authors, such as Brown et al. (2008), Caires et al. (2005) or Conyers et al. (2003) also found that, in the short term (2–4 years), the surface addition of $CaCO_3$ on NT plots raised

Table 4
Effect of tillage (T) and Ca-amendment (A) on soil acidity, exchangeable Ca and Al forms.

Depth	Treatment	PH	Ca ($cmol_c\ kg^{-1}$)	AlK ($cmol_c\ kg^{-1}$)	$Al_{Cu\text{-}K}$ ($cmol_c\ kg^{-1}$)	$Al_{Oxa\text{-}Cu\text{-}K}$ ($cmol_c\ kg^{-1}$)
0–5 cm						
	Tillage					
	TT	5.6	3.25	0.28	0.67	16.02
	NT	5.8	3.85	0.16	0.69	18.00
	Ca-amendment					
	Not amended	5.2	1.73	0.44	0.70	15.43
	Amended	6.1	5.87	0.00	0.66	18.60
	Effects					
	T	ns	*	*	ns	ns
	A	***	***	***	ns	**
	TXA	+	+	**	ns	*
5–10 cm						
	Tillage					
	TT	5.5	2.87	0.29	0.76	18.88
	NT	5.4	2.46	0.34	0.94	20.17
	Ca-amendment					
	Not amended	4.9	1.21	0.62	0.83	18.04
	Amended	6.0	4.12	0.00	0.86	21.01
	Effects					
	T	ns	*	ns	*	+
	A	***	***	***	ns	**
	TXA	ns	+	ns	ns	ns
10–25 cm						
	Tillage					
	TT	5.2	1.14	0.50	1.19	18.65
	NT	4.9	0.84	0.64	1.24	19.63
	Ca-amendment					
	Not amended	4.8	0.66	0.83	1.14	18.00
	Amended	5.3	1.32	0.31	1.28	20.28
	Effects					
	T	+	*	ns	ns	+
	A	***	***	***	ns	**
	TXA	ns	*	ns	ns	+
25–50 cm						
	Tillage					
	TT	5.0	0.71	0.88	nd	nd
	NT	4.9	0.82	0.86	nd	nd
	Ca-amendment					
	Not amended	4.9	0.52	0.96	nd	nd
	Amended	4.9	1.02	0.78	nd	nd
	Effects					
	T	ns	ns	ns		
	A	ns	*	ns		
	TXA	ns	ns	ns		

Al_K, exchangeable Al^{3+}; $Al_{Cu\text{-}K}$, Al-OM compounds with low to medium stability; $Al_{Oxa\text{-}Cu\text{-}K}$, amorphous Al plus the highly stable Al-OM compounds plus the Al from the octahedric layer of 2:1 clay mineral; TT, traditional tillage; and NT, no-tillage.
+ $p < 0.1$.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
ns, not significant; and nd, not determined.

pH and Ca contents down to a depth of 20 cm. In our case the content of active or easily soluble lime (21.3%) of the SF together with the high solubility of the RG probably extended the effect of the Ca-amendment to greater depths.

4.3. Forms of Al in the solid fraction and their relationships with organic carbon and GRSPs

KCl-extracted aluminium represents exchangeable Al^{3+}, which is typically used to calculate soil liming requirements (Juo and Kamprath, 1979). As shown in Fig. 2, Al_K changed in the opposite direction to pH and exchangeable Ca. In fact, Al_K was negatively correlated with pH ($r = -0.86^{***}$) and exchangeable Ca ($r = -0.87^{***}$) in the top two soil layers. In the 0–5 cm and 5–10 cm layers of the amended plots, the pH increase caused the precipitation of Al^{3+}, probably in the form of insoluble amorphous hydroxides (Haynes and Naidu, 1998), reducing the Al_K content to zero. In the 10–25 cm layer the decrease of Al_K was also due to the effect of the SF and the Ca supplied by the RG. In the deepest layer (25–50 cm), Al_K tended to decrease due to the effect of the Ca supplied by the RG (Shainberg et al., 1989), although the differences were not significant. Our results are in agreement with the processes reported by Kinraide and Parker (1987) and Noble et al. (1988), who studied the role of gypsum in the reduction of Al^{3+} activity. They also support previous findings from Peregrina et al. (2007) and Mariscal-Sancho et al. (2009), who demonstrated the effectiveness of RG for decreasing Al^{3+} activity in these soils.

With regards to the unamended plots, in the 0–5 cm layer we observed the lowest Al_K contents in the NT plots (0.32 $cmol_c\ kg^{-1}$) where both TOC and POC (36.8 g kg^{-1}, 13.7 g kg^{-1}) contents were significantly higher. On the other hand, the highest Al_K contents were observed in the TT plots (0.56 $cmol_c\ kg^{-1}$) which had the lowest organic carbon contents for both TOC and POC (31.5 g kg^{-1}, 9.7 g kg^{-1}). In this layer we also observed a strong negative

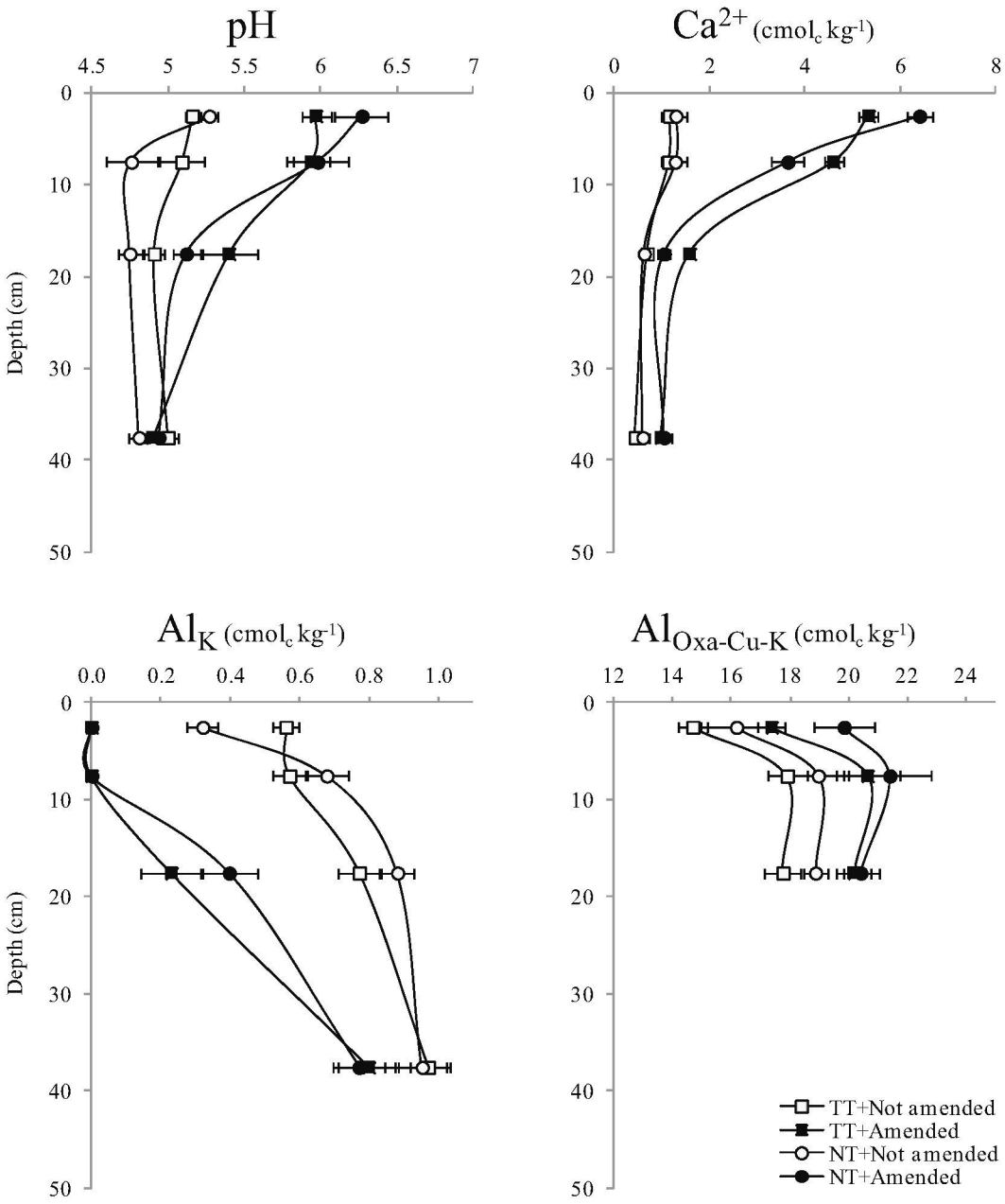

Fig. 2. Vertical profile of pH, exchangeable Ca, Al_K and $Al_{Oxa\text{-}Cu\text{-}K}$. TT, traditional tillage; and NT; no-tillage. Bars represent standard error of the mean.

correlation of Al_K with both POC ($r = -0.89^{***}$) and TOC ($r = -0.74^{***}$). Our results confirm the above-mentioned positive effect of SOM on the reduction of Al toxicity and the higher coefficient with POC suggests a more active role of this labile fraction in reducing Al toxicity. To our knowledge, there are no studies on the relationship between POM and the decrease of Al_K. The detoxifying effect of Al by SOM could be related to the complexation of the Al with decomposing residues. Since the POM is a labile easily-decomposed fraction of the SOM, this process would be enhanced by the greater POM content in the NT plots. Although the difference in pH between the NT and TT unamended plots was not significant, the increase of OH^-, as a result of chemical and biological decomposition (Wong and Swift, 2003) of POM could result in the precipitation of Al, which would also contribute to reducing aluminum toxicity.

Along the same lines, we also found a positive effect of the GRSPs, which were enhanced in the absence of tillage, on the reduction of Al^{+3} toxicity. In the two layers with protein measurements (0–5 and 5–10 cm), the reduction of the Al_K in the NT + not amended plots could also be attributed to the GRSPs effect, since we found an inverse relationship between Al_K and both GRSPs (see Fig. 3a and b), and more markedly with EE-GRSP ($R^2 = 68.8\%$) in the 0–5 cm layer (Fig. 3c). Our results support previous findings from Aguilera et al. (2011) who suggested that, in the absence of Ca-amendment, GRSPs immobilize some of the Al_K, thus reducing aluminium toxicity. Our results highlight the special role that EE-GRSP seems to play, although further studies are needed to properly understand the process.

With respect to $Al_{Cu\text{-}K}$, which represents the aluminium that forms compounds with SOM of low and medium stability, we observed no effect of the Ca-amendment. These results differ from those of Garrido et al. (2003), who looked at the effect of red gypsum and sugar foam separately on the top three horizons of a Plinthic Palexerult from our area under laboratory conditions and

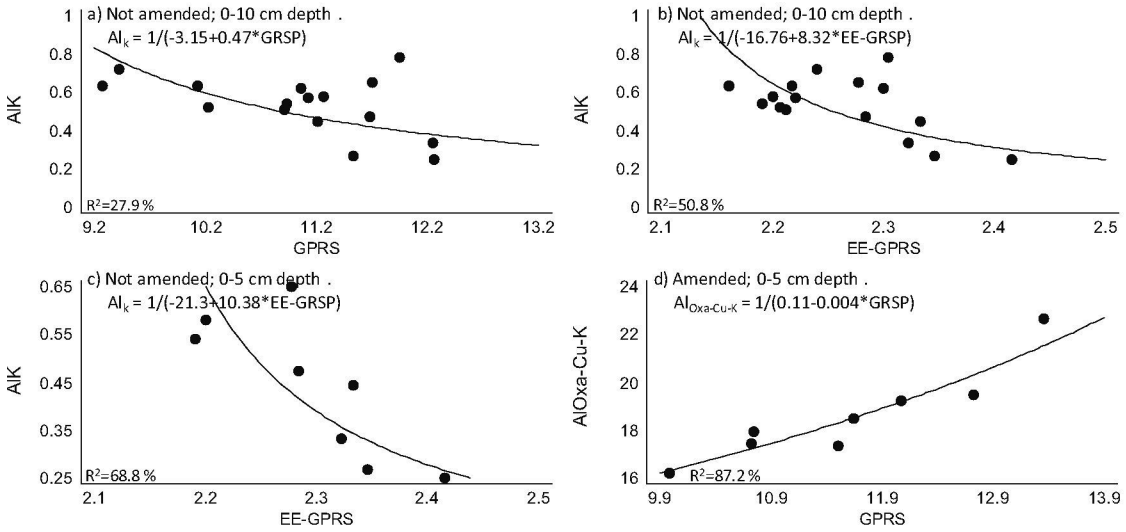

Fig. 3. Relationships between aluminium and glomalin-related soil proteins. (a) Al_K with total ($p = 0.05$) and (b) easily extractable glomalin-related soil proteins ($p = 0.02$) for both 0–5 and 5–10 depths in unamended plots; (c) Al_K and easily extractable glomalin-related soil proteins for 0–5 cm depth in unamended plots ($p = 0.02$) and (d) $Al_{Oxa-Cu-K}$ and total glomalin-related soil proteins for 0–5 cm depth in amended plots ($p = 0.01$).

observed that both amendments decreased Al_K and increased Al_{Cu-K}. They attributed the increase of Al_{Cu-K} to the lime, which caused a partial depolymerization of organic unhydrolysable compounds, which in turn facilitated the extraction of the Al bound to SOM by $CuCl_2$. However, this effect may be short-lived since Álvarez et al. (2009) observed an increase in the Al extracted with 0.5 M $CuCl_2$ during the first year of their study that turned into a decrease by the third year. This last result is consistent with the decrease in this form of Al with increasing pH reported by Godsey et al. (2007). In our work, we found a very weak inverse linear relationship ($r = -0.34^{*}$) between Al_{Cu-K} and pH when considering all soil layers together, which vanished, however, when considering each layer in isolation. The lack of a response of Al_{Cu-K} to Ca-amendment suggests a balance between the Al released from the SOM and the increasing ability of the organic matter to bind Al with increasing pH (Hargrove and Thomas, 1981b). With regards to tillage, we did not observe an effect in the 0–5 cm layer, whereas in the 5–10 cm layer the NT plots had a higher Al_{Cu-K} content compared with TT. According to Haynes and Mokolobate (2001) some of the Al-OM compounds are soluble and we suggest that some of them could have moved from the surface to the 5–10 cm layer.

In relation to the $Al_{Oxa-Cu-K}$, which represents the amorphous Al plus the highly stable Al-OM compounds and the Al from the octahedric layer of 2:1 clay minerals, concentrations considerably exceeded those of the other forms of Al (Al_K and Al_{Cu-K}). The prevalence of kaolinite in the clay fraction of these soils (Espejo, 1987) suggests that the Al coming from the octahedric layer of 2:1 clay minerals was very low. The effect of the Ca-amendment on $Al_{Oxa-Cu-K}$ was opposite to that on Al_K, with higher $Al_{Oxa-Cu-K}$ contents observed in the amended plots. A similar effect was observed by Álvarez et al. (2009) three years after liming and also by Garrido et al. (2003) in a laboratory experiment. The increase in $Al_{Oxa-Cu-K}$ in the amended plots is in agreement with the process described above: that Al^{3+} in the soil solution and the Al^{3+} displaced from the exchange complex by the Ca^{2+} is precipitated as amorphous hydroxides of aluminium as a result of the pH increase (Haynes and Naidu, 1998). The inverse linear relationship between $Al_{Oxa-Cu-K}$ and Al_K ($r = -0.71^{**}$), and the direct relationship between $Al_{Oxa-Cu-K}$ and pH ($r = 0.77^{***}$) in the top layer also supports this hypothesis. The latter relationship is especially marked in the NT + amended plots ($r = 0.99^{**}$). In addition, the strong relationship found in the surface layer of the amended plots between $Al_{Oxa-Cu-K}$ and the GRSPs, most marked with the GRSP ($R^2 = 87.2\%$; Fig. 3d), suggests that the $Al_{Oxa-Cu-K}$ includes non-toxic stable compounds of Al with the GRSPs. Bearing in mind the strong relationship between $Al_{Oxa-Cu-K}$ and GRSPs, together with the fact that NT increased the content of GRSPs, this could explain the higher content of $Al_{Oxa-Cu-K}$ in NT + amended plots compared with the TT + amended.

With regards to the relationship between organic carbon and $Al_{Oxa-Cu-K}$ we found a positive linear relationship with TOC ($r = 0.79^{*}$) and POC ($r = 0.71^{*}$) in the 0–5 cm layer of the unamended plots. Not tilling the unamended plots raised the correlation coefficient of $Al_{Oxa-Cu-K}$ with POC ($r = 0.79^{*}$) whilst weakening the relationship with TOC ($r = 0.91$, $p = 0.09$). These results, together with the decrease of the Al_K attributed to the SOM in the NT + not amended plots, suggest that the reduction of Al toxicity was more closely related to the formation of amorphous Al and highly stable Al-OM compounds ($Al_{Oxa-Cu-K}$) than to the Al-OM compounds with low and medium stability (Al_{Cu-K}). Since the differences in pH between the NT and TT unamended plots are not significant, the increase in $Al_{Oxa-Cu-K}$ in the 0–5 and 5–10 cm layers could be related more to the highly stable Al-OM compounds than to the Al in the form of amorphous hydroxides. This idea is also supported by the strong linear relationship between GPRS and $Al_{Oxa-Cu-K}$ ($r = 0.94$; $p = 0.05^{*}$) observed in the surface layer of the NT + not amended plots.

4.4. Water stable aggregates

The higher $WSA_{1-2\ mm}$ found in the 0–5 cm layer under NT compared with TT confirms that even in very stable soils, NT improves soil aggregation, as previously demonstrated by several studies in various environments (Six et al., 1999; Six et al., 2002; Madari et al., 2005; Fernández-Ugalde et al., 2009). We did not observe either a negative or positive effect of the Ca-amendment, possibly due to the opposing effects of the Ca^{2+} on soil aggregation mentioned above. Also, in coarse textured soils such as ours, aggregation depends more on SOM than on Ca^{2+} content (Berglund, 1971; Ledin, 1981). Contrary to Wuddivira and Camps-Roach (2007), who found an additive effect of Ca and SOM on $WSA_{1-2\ mm}$ in acid sandy and kaolinitic soils like ours, we found no improvement in aggregation when combining the Ca-amendment and NT. This is possibly because the positive effect of the addition

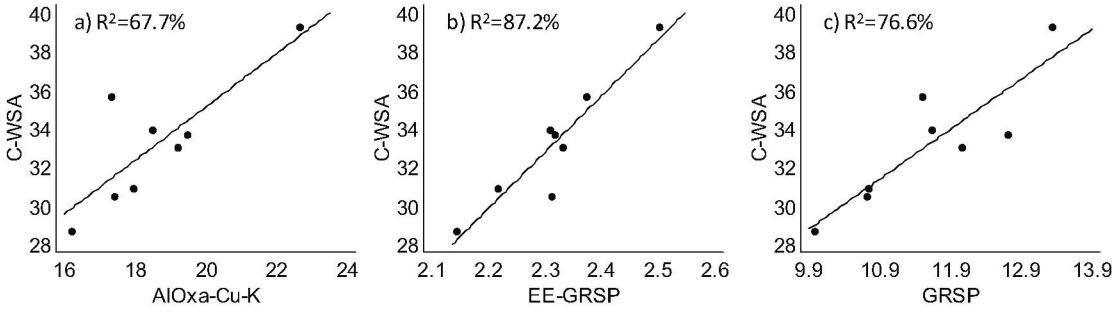

Fig. 4. Relationships of C-WSA with (a) $Al_{Oxa\text{-}Cu\text{-}K}$, (b) easily extractable and (c) total glomalin-related soil proteins for 0–5 cm depth of amended plots ($p < 0.01$).

of Ca^{2+} through bridging is expected only in the long term, as reported by Six et al. (2004). In addition, we found no direct relationship between $WSA_{1-2\ mm}$ and GRSPs, as reported by other authors (e.g., Wright and Upadhyaya, 1998). However, we found $WSA_{1-2\ mm}$ to be directly related to some variables that were increased by both the Ca-amendment and the absence of tillage in the surface layer: $WSA_{1-2\ mm}$ was positively correlated with TOC (0.67**) and POC (0.64**), confirming that in our coarse-textured soils, SOM plays an important role. In our soils, we expect a positive effect of the combination of Ca-amendment and NT on aggregate stability in the long term, as previously found by Briedis et al. (2012b) after 15 years in Brazilian soils using the distribution of aggregate classes. It is worth reconsidering the suitability of $WSA_{1-2\ mm}$ for describing soil aggregation in these stable soils. Perhaps the use of another measurement such as the distribution of aggregate classes may be more sensitive to changes in soil management.

Another parameter related to aggregate stability is C-WSA. As expected, we observed a higher C-concentration of stable macroaggregates compared with the unstable fraction. Similar results were found by Puget et al. (2000) who attributed them to the additional SOM binding microaggregates within stable macroaggregates. By contrast, the unstable aggregates were simple associations of microaggregates and primary particles, with no additional organic binding material. The positive effect of NT on C-concentration of stable macroaggregates has already been reported by a number of studies (e.g., Pinheiro et al., 2004; Mikha and Rice, 2004). Our results confirm that no-tillage not only increased the amount of stable aggregates, but also promoted their accumulation of carbon. Combining NT with the Ca-amendment did not result in higher carbon accumulation in the stable macroaggregates, suggesting that the extra TOC and POC present in the NT + amended plots is not protected within the stable aggregates, at least during the first four years. This carbon would, therefore be susceptible to mineralization.

In general, C-WSA exhibited stronger relationships with the studied parameters than $WSA_{1-2\ mm}$. In the 0–5 cm layer, C-WSA was positively correlated not only with TOC ($r = 0.63^{**}$) and POC ($r = 0.69^{**}$), but also with EE-GRSP ($r = 0.76^{***}$), GRSP ($r = 0.62^{*}$) and $Al_{Oxa\text{-}Cu\text{-}K}$ ($r = 0.61^{*}$), with the latter relationships being stronger in the amended plots. In these plots, the correlation coefficient with EE-GRSP was $r = 0.93^{***}$ (Fig. 4b), with GRSP it was $r = 0.98^{**}$ (Fig. 4c) and with $Al_{Oxa\text{-}Cu\text{-}K}$ it was $r = 0.82^{*}$ (Fig. 4a). The strong correlation between C-WSA and GRSPs allows us to hypothesize that a sizeable fraction of organic C in water-stable macroaggregates could be of fungal origin. The relationship with $Al_{Oxa\text{-}Cu\text{-}K}$ is in agreement with Barthès et al. (2008), who highlighted the importance of Al sesquioxides (extracted with ammonium oxalate) in the formation of stable macroaggregates and the protection of SOM in soils with low-activity clay in tropical soils.

Although we did not observe a direct relationship of $WSA_{1-2\ mm}$ with GRSPs or with $Al_{Oxa\text{-}Cu\text{-}K}$, their relationship with C-WSA suggests that they play a role in aggregate stabilization. This was confirmed by a multiple linear regression, with C-WSA as the dependent variable and all other factors that potentially play a role in soil aggregation (viz. TOC, POC, Ca, GRSPs, $Al_{Cu\text{-}K}$ and $Al_{Oxa\text{-}Cu\text{-}K}$) as independent variables. For the top two soil layers, EE-GRSP and $Al_{Oxa\text{-}Cu\text{-}K}$ explained 43% of the total variance, while including exchangeable Ca in the regression model only explained an additional 6%. In the 0–5 cm layer the variance explained by EE-GRSP and $Al_{Oxa\text{-}Cu\text{-}K}$ was even greater (63%), suggesting that both EE-GRSP and $Al_{Oxa\text{-}Cu\text{-}K}$ may be important for protecting organic carbon within stable aggregates in our soil.

5. Conclusions

After four years the Ca-amendment raised TOC and POC slightly in the 0–5 cm soil layer, although this effect vanished with tillage. We obtained the highest SOM contents at this depth by combining NT with the Ca-amendment, with the largest increase being in the POC fraction. Soil aggregation was enhanced by NT, but the Ca-amendment had no effect on aggregation, despite increasing TOC and POC. The protection of C within stable aggregates was closely related to $Al_{Oxa\text{-}Cu\text{-}K}$, which includes the stable Al-OM compounds, of which some are possibly formed with GRSPs.

Our results confirm the beneficial effect of SOM, especially the easily-decomposed POC fraction, and GRSPs, possibly through the formation of non-toxic stable compounds with the aluminium. NT helped to reduce Al toxicity by increasing both the SOM and the GRSPs.

The combination of NT with the Ca-amendment significantly improved TOC, POC, pH, Ca, Al_K, and $Al_{Oxa\text{-}Cu\text{-}K}$ in the 0–5 cm soil layer, without adversely affecting other soil parameters. From our findings we can conclude that managing soils like the one at the experimental site with NT plus a Ca-amendment appears to be the most suitable choice for ensuring sustainable production, through the accumulation of SOM and the improvement of chemical properties in acid soils, at least in the top layer.

Acknowledgements

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